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Chapter 2

The current state of human *in vitro* cardiac disease modeling: a focus on gene editing and tissue engineering

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ABSTRACT

Until recently, *in vivo* and *ex vivo* experiments were the only means to determine factors and pathways involved in disease pathophysiology. After the generation of characterized human embryonic stem cell lines, human diseases could readily be studied in an extensively controllable setting. The introduction of human-induced pluripotent stem cells, a decade ago, allowed the investigation of hereditary diseases *in vitro*. In the field of cardiology, diseases linked to known genes have successfully been studied, revealing novel disease mechanisms. The direct effects of various mutations leading to hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic cardiomyopathy, or left ventricular noncompaction cardiomyopathy are discovered as a result of *in vitro* disease modeling. Researchers are currently applying more advanced techniques to unravel more complex phenotypes, resulting in state-of-the-art models that better mimic *in vivo* physiology. The continued improvement of tissue engineering techniques and new insights into epigenetics resulted in more reliable and feasible platforms for disease modeling and the development of novel therapeutic strategies. The introduction of CRISPR-Cas9 gene editing granted the ability to model diseases *in vitro* independent of induced pluripotent stem cells. In addition to highlighting recent developments in the field of human *in vitro* cardiomyopathy modeling, this review also aims to emphasize limitations that remain to be addressed; including residual somatic epigenetic signatures induced pluripotent stem cells, and modeling diseases with unknown genetic causes.

INTRODUCTION

Heart failure is a clinical syndrome that is caused by a wide variety of factors and between 2011 and 2014 an estimated 6.5 million adults were diagnosed with heart failure¹. The number of heart failure patients is rising markedly. Dysfunctionality of the cardiac muscle leading to heart failure can be caused by different cardiomyopathies. The most common forms are hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), followed by arrhythmogenic cardiomyopathy (ACM) and left ventricular non-compaction cardiomyopathy (LVNC)²⁻⁵. They result from a complex and diverse mechanism that is often a mix of functional, structural, and biological adaptations specific for each cardiomyopathy. This makes studying heart failure pathophysiology a daunting task.

Technological advances that were made during the last decades enabled researchers to non-invasively study cardiac function in detail. Nevertheless, studying pathological molecular mechanisms occurring in the failing heart of patients primarily involves invasive methods. Taking any form of biopsy from cardiac tissue comes with the risk of perforation. The amount of material is often insufficient for extensive molecular analyses and biopsies are only taken in very few patients with severe (end-stage) cardiac pathology. Moreover, cardiomyocytes are non-proliferative, which makes *in vitro* culturing of primary cardiomyocytes complicated. Alternatively, standardized cell lines were used (e.g. H9C2, HL-1 or immortalized cardiomyocytes), while these cells proliferate indefinitely and resemble cardiomyocytes to some extent, each line also has major disadvantages (e.g. non-human cells or tumor-like properties). In addition, using animals to isolate (neonatal) cardiomyocytes requires a large number of animals to acquire sufficient amounts of cells.

The emergence of human embryonic stem cells (hESC) and the development of appropriate culturing techniques, quickly made them a potent tool to study previously rare tissues and mechanisms⁶. In 2007, the pioneering methods for generating human induced pluripotent stem cells (hiPSC) were published and provided the means to conduct patient-specific *in vitro* studies⁷. The development of these cell-based tools enabled researchers to attempt recapitulating various aspects of a disease through *in vitro* disease modeling.

This review aims to highlight the current status of *in vitro* cardiomyopathy models while focusing on tissue engineering and gene editing in order to recapitulate human cardiomyopathies.

CARDIAC DISEASE MODELING - TRANSLATION TO THE CLINICAL SETTING

Cellular sources for *in vitro* cardiomyopathy models

Early *in vitro* cardiac tissue models were based on either immortalized human cell lines or cells isolated from animals. The immortalized human ventricular AC16 cell line was developed using fusion of primary ventricular cardiomyocytes with an SV-40 transformed fibroblast cell line⁸. These cells resemble human cardiomyocytes to great extent (e.g. these cells contract and express main cardiac genes), but the proliferative capacity of these cells remains the main disadvantage as proliferating cardiomyocytes cannot maintain stable myofibrils.

Primary cardiomyocytes isolated from neonatal mice, rats, and chicken embryos were popular cell sources for *in vitro* cardiac models⁹⁻¹¹, but research based on these primary cells demonstrated that animal cell-based models cannot truly recapitulate human physiology. Consequently, more sophisticated cell models were developed to create human-like tissue models^{12,13}. However, establishing human models proved to be challenging as cardiac tissue or isolated cardiomyocytes from patients are difficult to obtain and cannot survive long-term culture¹⁴.

Human pluripotent stem cells

Cardiomyocytes were considered a rare cell type for *in vitro* studies, until hESC-derived cardiomyocytes (hESC-CM) were the first source of human heart cells for large scale experimental set-ups¹⁵. Since the introduction of hESC-CM in 2001, the use of hESC as a source for *in vitro* cardiac disease modeling has been copious¹⁶. Additionally, hiPSC-derived cardiomyocytes (hiPSC-CM) were found to recapitulate phenotypic characteristics caused by genetic variations¹⁷, which renders these cells a suitable source for human disease models. Furthermore, hiPSC-CM were found to be a powerful tool for patient stratification in regard to drug safety and responsiveness¹⁸. To date, artificially matured patient-derived hiPSC-CM proved to be similar in to isolated primary human cardiomyocytes molecular, mechanical, electrophysiological, metabolic, and ultra-structural properties^{19,20}. However, hiPSC-CM exhibit various fetal characteristics as opposed to mature (isolated) cardiomyocytes. To resolve these issues, hiPSC-CM can be cultured for extended periods or subjected to specific bioengineering approaches. Protocols using hormone stimulation¹⁹ or conditioning with mechanical stress and electrical pacing^{21,22} have collectively led to a more mature phenotype, but the exact mechanisms that induce maturation remain only partially understood²³⁻²⁶. Diverse epigenetic processes, including long-noncoding RNA (lncRNA)²⁷, microRNAs²⁸, chromatin and histone proteins²⁹, and DNA methylation²⁹ have been suggested as crucial mediators in both developmental processes and in disease.

INHERITED CARDIOMYOPATHIES - HIPSC TO MODEL GENETIC CAUSALITY

A plethora of genetic mutations have been associated with the pathogenesis of genetic heart diseases, including the main inherited cardiomyopathies (i.e. HCM, DCM, ACM and LVNC). Investigating how genetic mutations explain causality in the pathophysiology of cardiomyopathies and how they interact with secondary genetic and environmental factors is imperative to improving diagnosis and decision-making regarding treatment strategies. The introduction of patient-specific hiPSC-CM provides a versatile new tool that may tremendously improve our understanding of the disease mechanisms. Consequently, these cells have been widely applied to study the complexity of cardiac disease. However, cardiomyopathies are divided into four classes, each with a distinct pathophysiology, resulting in various types of heart failure. The most common cardiomyopathy, HCM, is characterized by increased cardiac mass due to left ventricular wall thickening (hypertrophy) that most often is asymmetric, with particular involvement of the interventricular septum, myocytes disarray, and cardiac fibrosis³⁰. DCM is characterized by left ventricular chamber enlargement and systolic dysfunction, which often leads to heart failure, arrhythmia, and sudden death. ACM predominantly affects right ventricular cardiomyocytes and occurs due to defects in the cardiac desmosome as a consequence of mutations in key desmosomal components, but also due to ion channel defects. Consequently, ACM hallmarks include right ventricular dilation, scarring, exaggerated lipogenesis and lipid infiltration, and arrhythmias. Finally, LVNC is characterized by cardiac non-compaction, primarily resulting in trabeculation and deep recesses in the left ventricle. Many studies performed in patient-derived hiPSC-CM have often recapitulated these respective hallmarks of inherited cardiomyopathies and thereby markedly increased our understanding of underlying molecular mechanisms, as summarized in [Table 1](#). In addition to cardiomyopathies, inherited arrhythmias are generally caused by a pathological mutation in a gene encoding an ion channel or an associated protein. However, this review focusses on cardiomyopathies, whereas arrhythmias are beyond the scope of this review. A recent review highlights the recent advances in the use genome editing to study cardiotoxicity and model inherited arrhythmia³¹.

DISEASE MODELLING UTILIZING KNOWN VS. UNKNOWN GENETIC VARIATIONS

In vitro disease modeling has proven to be a valuable tool to study molecular pathophysiological mechanisms and disease etiology for diseases with a known genetic cause. Indeed, modeling disease without a known genetic defect is challenging. Nevertheless,

Table 1 - Summary of cardiomyopathy-associated mutations that have been studied in hiPSC-based *in vitro* models.

	Gene	Mutation	Main phenotype	Ref	
HCM	MYH7	p.R442G	Enlarged cellular size, disorganized myofibrils, disrupted sarcomere structure, dysfunctional ion channel homeostasis.	32	
		p.R663H	Enlarged cellular size, contractile arrhythmia, dysfunctional Ca ²⁺ -handling, increased [Ca ²⁺] _i	33	
	MYBPC3	c.1358-1359insC	Enlarged cellular size, disrupted gene expression profile	34	
		p.Q1061X	Enlarged cellular size, aberrant electrophysiological properties, dysfunctional Ca ²⁺ -handling, and disrupted gene expression profile	35	
		p.G999-Q1004del	Enlarged cellular size, disorganized myofibrils	36	
		c.2373dupG	Aberrant electrophysiological properties, reduced contractile force generation, aberrant bioenergetics	37	
	TPM1	p.D175N	Enlarged cellular size, aberrant electrophysiological properties, dysfunctional Ca ²⁺ -handling, disrupted gene expression profile	35	
	DCM	TTN	p.A22352fs+/- p.P22582fs+/- p.W976R+/-	Reduced contractile force generation, disrupted sarcomere structure, impaired response to mechanical and β-adrenergic stress	38
LMNA			p.R225X p.Q354X p.T518fs	Nuclear blebbing, increased senescence, increased apoptosis	39
			TNNT2	p.R173W	Dysfunctional Ca ²⁺ -handling, reduced contractile force generation, disrupted sarcomere structure
DES		p.A285V	Disrupted sarcomere structure, ultrastructural disarray	42	
RBM20		p.R636S	Sarcomeric remodeling, dysfunctional Ca ²⁺ -handling, increased [Ca ²⁺] _i , disrupted gene expression profile	43,44	
PLN		p.R14del	Dysfunctional Ca ²⁺ -handling, aberrant electrophysiological properties, increased hypertrophy markers	45,46	
ACM		PKP2	c.2484C>T c.2013delC	Increased lipogenesis, increased apoptosis, dysfunctional Ca ²⁺ -handling, disrupted desmosome structure	47
			c.1841T>C	Increased lipogenesis, disrupted desmosome structure	48
	c.972InsT/N		Increased lipogenesis, disrupted desmosome structure	49	
	SCN5A	p.R1898H	Dysfunctional Na ⁺ -handling	50	
LVNC	TBX20	c.951C>A	Reduced proliferative capacity, disrupted gene expression profile	51	

these *in vitro* models have also been successfully applied to cardiac disease that develop without a known causing genetic variant. For example, BurrIDGE et al. have recently demonstrated that it is possible to determine the underlying genetic aberrations found in heart failure patients that experienced doxorubicin-induced cardiotoxicity⁵². Furthermore, hiPSC were used to screen for cardiovascular toxicity of anticancer tyrosine kinase inhibitors using multiple healthy controls and two patients receiving cancer treatment⁵³. Additionally, studies have identified genetic targets in hypoplastic left heart syndrome in hiPSC with previously unknown mutations^{54,55}. These examples show that the use of hiPSC for *in vitro* cardiac disease modeling without the presence of a known genetic defect are

thus challenging, albeit not impracticable. This has also been demonstrated for other fields of disease, where hiPSC have been used to model non-cardiac diseases like sporadic Alzheimer's disease⁵⁶, chemotherapy induced neurotoxicity⁵⁷, and were shown a valuable tool in cancer research and precision oncology⁵⁸.

In vitro modeling of multifactorial diseases that are mechanistically complex or diseases that arise due to environmental causes is challenging and unrealistic. HiPSC are patient-derived and harbor all relevant genetic factors that may contribute to the disease. Hence, even when the exact underlying mechanisms of a disease are unknown, hiPSC provide a reliable platform for disease modeling. A disease of the heart is often assumed to arise from cardiomyocytes themselves. However, due to tightly regulated cell-autonomous versus non-cell-autonomous responses (e.g. interactions between cardiomyocytes and neighboring fibroblasts and endothelial cells), this may not be the case. A disease may very well originate in a non-myocyte cell type and functionally disrupt cardiomyocyte function (for example: endothelial dysfunction and subsequent disturbed perfusion). As hiPSC can differentiate towards virtually all cell types, researchers can quickly change protocols and obtain these other relevant cell types based on hiPSC derived from a single patient. This potential of plasticity highlights the significance of hiPSC as a platform for disease modeling.

GENE EDITING IN CARDIOMYOPATHIES

Traditional genome editing methods have been mostly based on zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). Both ZNFs and TALENs use DNA binding motifs that can be designed and combined to target any nucleotide sequence for cleavage. ZNFs target trinucleotide sequences, while TALENs can recognize a single nucleotide. This makes the use of TALENs generally more straightforward. Recent technological breakthroughs for targeted gene editing using site-specific nucleases primarily related to clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) systems allow for genome engineering, reverse genetics, and targeted transgene integration experiments that can be performed in an accurate and reproducible fashion⁵⁹. The CRISPR/Cas9 system is based on site targeting based on guide RNA design and results in improved efficiency compared to earlier methods^{60,61}. Furthermore, site targeting is more flexible with the CRISPR/Cas9 system than with ZNFs and TALENs and offers the possibility to introduce multiple mutations at the same time by injecting different guide RNAs. By applying these tools, genes have been functionally removed from specific loci, thereby creating disease-causing mutations in hiPSC-CM or other cardiovascular disease models *in vitro*⁶². Vice versa, genetic mutations could be

corrected in patient-derived cells, resulting in the generation of an isogenic control cell line by exclusively eliminating the disease-causing genetic variation.

Correcting or silencing a pathological genetic variant can be used to develop future therapies. However, when applying this to human cardiomyopathies, many different, site-specific corrective strategies need to be designed and tested. This feat is challenging from a clinical trial and regulatory perspective. Each antisense oligonucleotide or guide RNA can only target a very specific nucleotide sequence and is therefore useful for a very small number of patients, which makes placebo-controlled trials, the regulatory standard, nearly impossible. This has prompted the evaluation of the possibility of broader genetic therapeutic avenues that can target normal genes to enhance cardiac function. For example: gene therapy (i.e. induced overexpression) has been applied to upregulate SERCA2a and as a result enhance myocardial contraction in heart failure patients with reduced ejection fraction.⁶³⁻⁶⁵ However, with respect to disease models, various studies have been successful in recapitulating specific diseases *in vitro* as well as reverting disease phenotypes by correcting a genetic variant as presented in **Figure 1**. These studies have been summarized in **Table 2**.

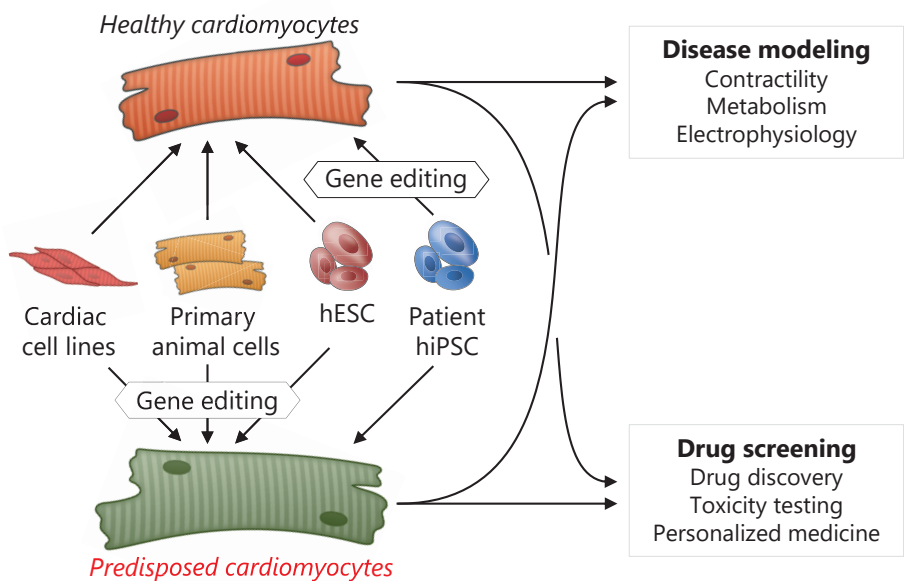


Figure 1 - Schematic representation of cell types as a basis for human *in vitro* models. Primary cells, cell lines and stem cells can be utilized as a basis for *in vitro* disease models to study cardiomyopathies. State-of-the-art gene editing techniques allow for the introduction of specific disease-causing mutations. Alternatively, gene editing can also be harnessed to generate isogenic control lines from patient-derived cells.

Table 2 - Studies that have generated in vitro disease models and studies that have repaired and rescued in vitro disease phenotypes.

	Gene	Mutation	Strategy	Ref
Gene repair	SCN5A	p.R1898H	CRISPR/Cas9-mediated gene repair	66
	PRKAG2	c.905G>A (p.R302Q)	CRISPR/Cas9-mediated gene repair	67
	PRKAG2	p.R302Q	CRISPR/Cas9-mediated gene repair	68
	DMD	Exon 3-6 del	CRISPR/Cas9-mediated exon deletion	69
	CALM2	p.D130G	CRISPR interference	70
	CALM2	p.N98S	CRISPR/Cas9-mediated allele knock out	71
Introduction of mutation	ADRB2	Multiple	PiggyBac-mediated gene editing	72
	GRK5	c.122A>T		
	RYR2	c.6737C>T		
	ACTC1	c.301G>A		
	TNNT2	p.I79N	CRISPR/Cas9-mediated gene editing	73

Generation of hESC-based disease models

While hiPSC are currently a popular choice for many cell-based studies, recent advances in the CRISPR-Cas9 technology have rendered hESC a valid and feasible alternative as well. Any wildtype cell can be altered to harbor a specific mutation using CRISPR-Cas9 mediated gene editing. Indeed, CRISPR-Cas9 can be applied to create the perfect experimental controls in hiPSC and hESC: a pathogenic mutation can be corrected in patient-derived hiPSC, while a putative pathogenic mutation can be inserted in otherwise wildtype hESC. As result, genetically edited stem cells are the same as their original cell line in all aspects except the edited genes. It is important to note that any method facilitating gene editing can result in off-target effects in various genomic regions. Following its introduction, studies demonstrated that this was also relevant for CRISPR-Cas9^{74,75}. However, in recent years, new nucleases have been discovered and have been verified to induce no off-target effects⁷⁶⁻⁷⁸. These new techniques allow for the generation of edited cell lines from a single source that only differ in the edited gene. This way, difference found between those cell lines can directly be attributed to a single mutation and can then be further studied in more complex models (e.g. patient-derived hiPSC-based models with familial controls).

Epigenetics and environmental influence

In contrast to a disease resulting from genetic variants, diseases can also arise from environmental factors, such as malnutrition, drug-related effects, exogenous toxins or maternal disease during gestation⁷⁹⁻⁸⁵. Some of these environmental factors can lead to epigenetic changes, like DNA methylation. In this case, chances of obtaining a phenotype will be extremely small in a hiPSC-based experimental setup. During reprogramming of somatic cells to hiPSC, most epigenetic features characteristic for a specific cell type are removed while cell type-specific marks remain⁸⁶. More specifically, every cell type has a

unique DNA methylation pattern. Importantly, epigenetic profiles that are linked to disease progression are lost during reprogramming. While losing disease-causing epigenetic marks due to reprogramming may result in a model without a phenotype, which directly emphasizes the need to focus on (and possibly attenuate) the epigenetics factors in a specific patient⁸⁷.

To conclude, the patient-derived aspect of hiPSC-based disease models enables studies to be designed that may unravel pathological mechanisms caused by genetic as well as epigenetic anomalies. Due to the precision with which all other (*in vitro* and *in vivo*) models are designed, it can be expected that not all disease-causing factors, e.g. DNA methylation, are included and are therefore overlooked.

CARDIAC TISSUE ENGINEERING

The heart is a complex organ composed of various cell types (e.g. cardiomyocytes, fibroblasts and endothelial cells) in a three-dimensional (3D) organization. While many studies are performed with two-dimensional (2D) *in vitro* cultures, previous studies showed that cells better recapitulate *in vivo* physiology when cultured in a 3D system^{88,89}. Additionally, generation of cardiac tissue containing an appropriate mix of cell types improved feasibility of studies that were previously challenging, such as studies involving electrophysiology, cell-cell or cell-extracellular matrix (ECM) interactions, co-cultures, or drug screening⁹⁰. Subsequently, it provides an adaptable platform with the ability to replace various animal-based studies, ultimately reducing the number of laboratory animals. To achieve such tissues for cardiac disease modeling, various techniques have been employed. Seminal work by Moscona in 1959 demonstrated that embryonic chicken cardiomyocytes spontaneously form beating cardiospheres. This was the basis for the currently most commonly used and adapted model: the engineered heart tissue model^{91,92}, where hESC-CM are seeded in a hydrogel. The hydrogel matrix is casted in a mold, which can be cultured under mechanical strain between fixed anchoring points⁹². The effects of various growth factors, cyclic uni- or multi-axial mechanical stretching, cardiomyocyte maturation, and electrical pacing⁹³ was studied using this model. The finding that non-myocytes promote contractile force generation while also better reflecting the composition of the human heart, compared to tissue consisting of purified cardiomyocytes, has led to the standardization of adding various non-myocytes to the tissue⁹⁴.

A second model of engineered cardiac tissue, is based on the same principle demonstrated by Moscona in which various cell types can aggregate into spheroids (or microtissues) under the right conditions. Non-adhesive surfaces, hanging droplets and rotation systems are used to generate spheroids⁹⁵. While spheroids are generally small and challenging to physically manipulate, they are very suitable to study 3D behavior of

cells and cell-cell interactions, drug testing, and can be used as building blocks to create larger tissues⁹⁶. A third and alternative approach to make tissues is the formation of cell sheets. By utilization of a coating that dissolves at room temperature, intact detached cell-monolayers that can be stacked to create thicker tissues for transplantation or drug screening⁹⁷.

However, the main limitation to these methods is the lack of vascularization and consequently low perfusion of oxygen and nutrients. Prefabricated channels and tubes have been incorporated in tissue constructs to address to improve tissue perfusion⁹⁸. As opposed to using self-assembly and artificial matrices as a basis for tissue engineering, decellularized explanted hearts were also demonstrated to be viable scaffolds⁹⁹. Although the main goal was to create fully functional hearts for transplantation, this has been largely unsuccessful to date. However, decellularized tissues retain hierarchical large and smaller vascular structures¹⁰⁰. These studies have set a precedent to use decellularized explanted tissues (i.e. small pieces of tissue) as a scaffold for tissue engineering. Remarkably, this can also be done with plant-derived scaffolds, as was recently demonstrated by Gerschlak et al¹⁰¹. The overarching goal is to develop a high throughput screening platform with highly representative cardiac tissue. As mentioned before, there have been many advances in this field recently. To reach this goal there have been various seminal studies published recently. The study by Mills et al. has elegantly demonstrated a procedure to generate high throughput screening platform based on human cardiac organoids¹⁰². Additionally, to induce maturation in these organoids, Mills et al. have activated the proliferation pathways mediated by β -catenin and Yes-associated protein 1 (YAP1). As a result, matured human cardiac organoids can be applied for high throughput screening. Alternatively, Foo et al. have recently introduced a method for the generation of vascularized cardiac tissues by transplanting human stem cell-derived cardiac precursors sub-capsularly onto kidneys in mice¹³. Furthermore, Lind et al. demonstrated that the popular “Heart-on-a-chip” concept can now be obtained by a combination of a 3D printed flexible chip and tissue engineering¹⁰³. These state-of-the-art tissue engineering techniques are summarized in [Figure 2](#).

CONCLUSIONS AND FUTURE PERSPECTIVES

In summary, to study a disease with incredible detail, target cells from various sources can be collected and cultured in 2D or 3D. These *in vitro* cultures can be manipulated very precisely, allowing researchers to pinpoint key factors of disease origin and progression. Building on these findings, novel drugs can be discovered and tested, driving the progression towards personalized medicine ([Figure 3](#)).

Depending on the field of study, *in vitro* disease models can be based on any cell type and source. However, in order study cardiomyocytes, the cell sources are largely limited

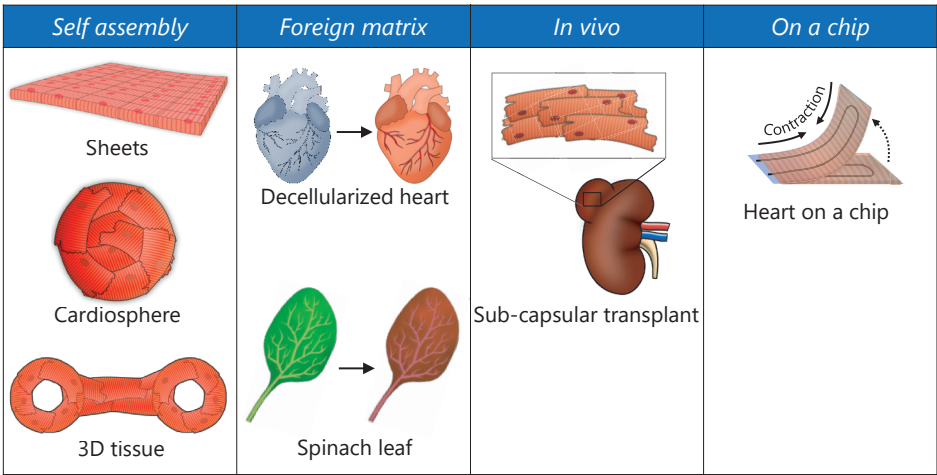


Figure 2 - Summary of different technical approaches to cardiac tissue engineering. Cardiac tissues can be generated by allowing cardiac cells to spontaneously form a tissue by self-assembly. Other approaches include the introduction of a decellularized matrix as a basis for reconstituted cardiac tissue, injecting human cardiac precursor cells into the murine kidney and machine-guided generation of cardiac tissue on a chip.

to pluripotent stem cells. An argument against the use of hiPSC is the residual epigenetic landscape that remains after reprogramming of any somatic cell type to hiPSC. Indeed, hiPSC can be cultured in pluripotent states similar to hESC and can be differentiated to virtually any cell type, but the effects of these residual epigenetic marks are unknown and depend to great extent on the source. This is a strong argument to use edited hESC instead of patient-specific hiPS cells, especially since each patient-derived cell line has a very different genetic background from any other hiPSC line. Therefore, a familial control has to be used for every patient line, as was indicated by Matsa et al¹⁸. In contrast, a single well defined hESC line (e.g. H9, H1, or HUES9) can be used as a basis for studies based on known mutations in which the unedited line can be a control for all introduced mutations.

Diseases often manifest as the result of one or multiple organs failing with a complex pathophysiology. A single organ contains various cell types with different functions, which often makes studying a disease challenging. By using *in vitro* disease models, it is possible to study specific cell types, study co-cultures of involved cell types, and manipulate tightly regulated mechanisms. Consequently, this approach disregards confounding factors and all systemic effects (i.e. inter-organ signaling) as seen with *in vivo* models. In contrast, this also entails that every aspect of the *in vitro* culturing method must be optimal for the specific model to be representative. Ultimately, it is no longer a near-impossible task to recapitulate patient-specific cardiomyopathies *in vitro*. As described in this review, recent technological advances have paved the way to more accessible culturing and engineering

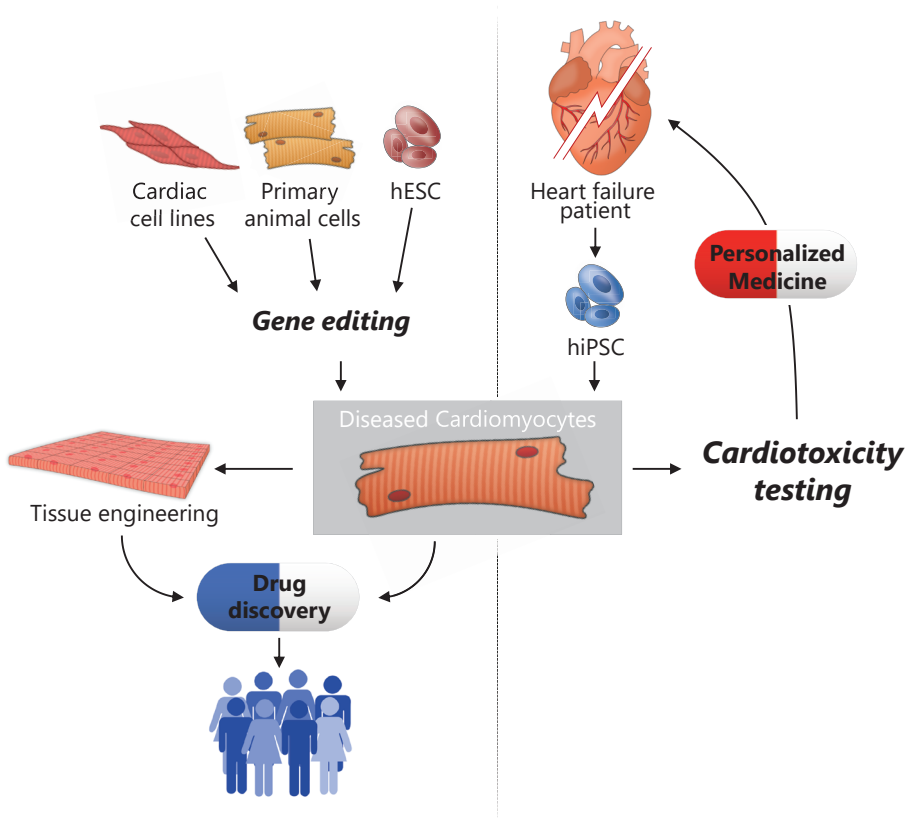


Figure 3 - Graphical summary. Cardiomyocytes generated from various sources can be used for in vitro disease modeling. Tissue engineering provides a means to obtain representative cardiac tissues that may be applied for high throughput screening of novel therapeutic strategies. In contrast, cardiomyocytes can be generated in a patient-derived manner, which allows for in vitro validation of personalized medicine to treat specific and rare diseases.

methods that will drive the field towards crucial insights into disease mechanisms and treatment options.

Presumably safe drugs have been withdrawn from the market more than once due to toxic effects in patients that were unobserved in the respective animal studies. Reasons vary from false negative results to off- and on-target toxicity (including unexpected cardiotoxicity). Typically, drug safety assessment and efficacy testing are performed in animal models followed by expensive clinical trials. To make drug discovery and testing more cost-effective, it is imperative that reliable alternative strategies are developed; human *in vitro* disease modelling will improve this process greatly.

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